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Essential fatty acids in breast milk of atopic mothers: comparison with non-atopic mothers, and effect of borage oil supplementation

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Objective: To evaluate whether levels of n-6 long chain polyunsaturated fatty acids (LCPs) in human breast milk are related to the mother's atopic constitution, and whether a decreased level can be restored by gamma-linolenic acid supplementation.
Design: Cross-sectional study and dietary supplementation trial.
Subjects: 20 atopic mothers and 20 non-atopic mothers (controls), all lactating.
Setting: General population.
Interventions: The atopic mothers were randomly assigned to low ($n = 10$) or high ($n = 10$) dosage oral supplementation with oral borage oil for one week (230 or 460 mg gamma-linolenic acid (18:3n-6) per day).
Main outcome measures: Essential fatty acid composition of the breast milk total fat fraction, determined by gas liquid chromatography.
Results: Arachidonic acid (20:4n-6) was lower in breast milk of atopic mothers compared with non-atopic mothers (0.39 wt% *vs* 0.46 wt%, difference −0.07% wt% (95% confidence limits −0.13, −0.01 wt%; $P < 0.05$). The ratio between linoleic acid and the sum of n-6 derivatives did not differ between these groups, indicating no difference in delta-6-desaturase (D6D) activity. Supplementation of the atopic mothers significantly increased the levels of gamma-linolenic acid and dihomo-gamma-linolenic acid in breast milk in a dose-related way, but the level of arachidonic acid was not increased.
Conclusion: We found a decreased level of arachidonic acid in breast milk in atopic compared to non-atopic mothers, but no indication that the rate-limiting enzymatic step (D6D) is involved. Supplementation increased the precursor pool but did not restore the level of arachidonic acid. We conclude that atopy is related to a metabolic disturbance beyond the D6D enzymatic step. A low level of arachidonic acid in breast milk may be a risk factor for the development of atopy in the infant, especially when the possible underlying metabolic disturbance of EFA metabolism is inherited by the child.
Sponsorship: F Hoffman-La Roche (Basel, Switzerland) and Friesland Dairy Foods (Leeuwarden, The Netherlands).
Descriptors: milk; human; hypersensitivity; fatty acids; essential; gamma-linolenic acid; arachidonic acid; dietary supplements
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Introduction

Disorders like atopic eczema, allergic asthma and allergic rhinitis are frequent manifestations of the atopic syndrome ('atopy'). One of the mechanisms possibly involved in the development of atopy is an abnormal metabolism of n-6 long chain polyunsaturated fatty acids (n-6 LCPs) (Manku *et al*, 1982; Melnik & Plewig, 1992). Linoleic acid (LA, 18:2n-6) from the diet is converted into arachidonic acid (AA, 20:4n-6) via the successive intermediates (gamma-linoleic acid (18:3n-6) (GLA) and dihomo-gamma-linoleic acid (20:3n-6) (DGLA). Several studies have shown abnormal n-6 fatty acid levels in patients with atopic eczema: decreased levels of n-6 LCPs in serum (Brown & Hansen, 1937), plasma (Manku *et al*, 1982; Wright & Sanders, 1991), red blood cells and mononuclear cells (Lindskov & Holmes, 1992), and adipose tissue (Wright & Sanders, 1991), sometimes also with slightly increased LA levels. This may indicate that atopic patients have a reduced conversion of linoleic acid to its LCP derivatives (Manku *et al*, 1982; Melnik & Plewig, 1992), possibly caused by an impaired activity of the rate limiting enzyme D6D, which converts LA to GLA (Manku *et al*, 1982). Derivatives of LA, notably DGLA and arachidonic acid (20:4n-6) (AA), are precursors of prostaglandins and leukotrienes, and these may be involved in the early maturation and differentiation of T-lymphocytes (Melnik & Plewig, 1992; Fulton & Levy, 1981; Tilden & Balch, 1982; Fischer *et al*, 1985).

Breast feeding has a protective effect on atopic eczema in high-risk infants (Kramer, 1988; Chandra, 1991; Saarinen & Kajosaari, 1995), even beyond childhood (Saarinen

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Contributors: CT was project leader, responsible for design and organization of the study, supervision of the field work and data analysis, and completion of the manuscript. AvH was responsible for supervision of laboratory analyses. IP was a research fellow and carried out field work, analysis and preparation of the manuscript. AM is a dietician and carried out field work. PvdB supervised the project.
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& Kajosaari, 1995). Long-chain polyunsaturated fatty acids (except the parent fatty acids linoleic acid and alpha-linoleic acid; LCPs) in human milk could play a protective role: human milk contains significant amounts of LCPs, whereas most term infant formulas are devoid of it (Melnik & Plewig, 1992). Businco *et al* (1993) and Wright & Bolton (1989) reported reduced levels of n-6 LCPs in breast milk from mothers of children who got atopic eczema. Since these studies were performed in a mixed group of atopic and non-atopic mothers, it was not clear whether the findings indicate a direct (causal) effect, or merely reflects the mothers' atopic constitution. The first aim of our study was therefore to examine the fatty acid composition of breast milk in relation to the mother's atopic constitution.

Human milk fatty acid composition is dependent on dietary intake (Harzer *et al*, 1984). Therefore, our second aim was to assess whether decreased levels of n-6 LCP in human milk of atopic mothers can be restored by means of dietary supplementation with GLA, thus bypassing the supposed rate limiting step from LA to GLA.

Methods

Participants

Participants were lactating mothers of full-term infants who breast-fed their infants exclusively or almost exclusively (at most one additional bottle feeding per day). They were recruited via midwives, infant welfare centers, and advertisements in regional newspapers.

Candidates were interviewed by telephone about atopic symptoms. A questionnaire on atopy was developed and validated for this purpose (van Bokhoven *et al*, 1999). Inclusion criteria for atopic mothers were a history of allergic asthma or rhinitis (at least two of a list of asthma and rhinitis complaints; related to exposure to pets, house dust or pollen or to blossoming of certain trees or grass; and onset before age 16 y), or atopic eczema (with typical localization, excluding contact allergy and urticaria, and onset before age 6 y), or history of positive allergy test, or improvement of any above-mentioned complaint with use of antihistamine drugs. Non-atopic mothers were included if they had none of these conditions, nor signs of urticaria or food allergy, nor a family history of atopy, nor a positive result on an allergy test in the past (if performed). Twenty atopic mothers and 20 non-atopic mothers entered the study.

Sampling and laboratory methods

Breast milk samples were collected between 8.00 am and 2.00 pm at home. After the child had finished drinking from one breast, a small amount of foremilk of the other breast was collected by the participant, either by manual expression or by using a mechanical or electric breast pump. Blood samples were taken between 10.00 am and 2.00 pm. Milk samples were preserved in tubes containing BHT in methanol; blood samples were preserved with EDTA. All samples were transported on ice. IgE was measured in the blood sample from the first visit, using a chemiluminometric immunoassay (Magic Lite SQ Allergy System, ALK-Abello, Horsholm, Denmark).

Fatty acid analysis

After separation from red blood cells by centrifugation, plasma was stored in tubes closed under nitrogen, at $-80^{\circ}C$ until analysis. Milk samples were stored at $-50^{\circ}C$. Total lipid extraction was performed as described by Bligh & Dyer (1959). L-a-dinonadecanoyl lecithin (PC 19:0) for plasma phospholipids and trinonadecanoin (TG 19:0) for breast milk were used as internal standards for quantification of the amounts of fatty acids.

Plasma phospholipid fractions were isolated by solid-phase extraction according to Kaluzny *et al*, 1985, after which they were hydrolyzed, and their fatty acids methylated with boron-trifluoride in methanol (Morissen & Smith, 1964). Fatty acid composition of plasma phospholipids and breast milk total lipid fraction was determined by gas liquid chromatography using capillary column (CP SIL 5, Chrompack®, Middelburg, The Netherlands). Results are reported as weight percent (wt%) of total fatty acids, computed as described by Al *et al* (1995).

As a measure of conversion of LA to its derivatives (and therefore delta-6-desaturase (D6D) activity), the ratio of LA to its LCP derivatives (excluding direct elongation products; LA/Σn-6LCPs) was calculated.

Supplementation

In the atopic group the effect of GLA supplementation on the fatty acid profile of breast milk and maternal blood plasma was studied. Before supplementation, milk and blood samples were collected twice, 1 week apart, to ensure completion of follow-up (run in) and to reduce intra-individual variance. Borage oil (from the seeds of *Borago officinalis*; Starflower oil, F. Hoffmann-La Roche, Switzerland) was used as a source of GLA. Borage oil contains 37% LA, 23% GLA, 15% oleic acid, and 25% other (mainly saturated) fatty acids. The atopic mothers were randomly divided into two groups: the low dose group received four capsules of 250 mg per day, containing 230 mg GLA in total; and the high dose group four capsules of 500 mg per day, containing 460 mg GLA in total. The capsules were taken spread over the day during one week. On day 8 a third sample of blood and milk was collected.

Statistical analysis

In the atopic group, the mean of the first and second measurements of each fatty acid was used for analysis. Comparison between the atopic and non-atopic group was done with Student's *t*-test; the effect of supplementation was measured using Student's *t*-test for paired samples. Two-sided 95% confidence limits are reported throughout.

Results

Baseline characteristics of mothers and infants are shown in Table 1. Fourteen of the 20 atopic mothers had a raised total IgE level (> 50 IU/ml), against 5 of the 20 non-atopic mothers. The groups were comparable with regard to other baseline variables.

In breast milk, AA was lower in atopic mothers compared with non-atopic mothers (Table 2). Also DGLA was somewhat lower, but the difference did not reach statistical significance. The ratio of LA to its LCP derivatives (LA/Σn-6LCPs) was similar in both groups. In plasma phospholipids no statistically significant differences were noticed (data not shown).

Table 3 shows the fatty acid composition in breast milk before and after supplementation. GLA and DGLA levels increased in a dose-dependent way, but AA did not change. The relative increase of GLA level in the low and high dose

Table 1 Baseline characteristics of mothers and infants in the non-atopic group and in the high-dose and low-dose supplementation atopic groups

Characteristic	*Non-atopic group (n=20)*	*Atopic group*	
		High dose (n=10)	*Low dose (n=10)*
Atopic history; number of subjects			
allergic asthma		7	4
allergic rhinitis		9	9
atopic eczema		5	3
family history of atopy		7	9
positive allergy test in the past		9	8
Plasma total IgE of mothers; number of subjects			
< 50 IU/ml	15	4	2
50–100 IU/ml	2	0	3
100–200 IU/ml	1	4	0
> 200 IU/ml	2	2	5
Age (y) of mothers; mean (range)	30 (25–38)	29 (22–35)	31 (27–35)
Pre-pregnant body mass index (kg/m^2) of mother; mean (range)	23 (18–31)	24 (20–29)	24 (20–32)
Number of children			
1	11	6	5
2–5	9	4	5
Duration of lactation (weeks post-partum); mean (range)	5 (2–12)	5 (2–11)	5 (2–10)
Birth weight (kg); mean (range)	3.5 (2.8–5.0)	3.3 (2.7–4.0)	3.5 (2.7–4.0)

group was +55% and +111% from baseline, respectively, and for DGLA this was +29% and +42%, respectively. The sum of n-6 LCPs was raised by +17% and +25%, respectively. Accordingly, the ratio of LA to its LCP derivatives (LA/Σn-6LCPs) was decreased by 12% and 21%, respectively. n-3LCPs were not consistently changed (data not shown). LCP changes in plasma phospholipids paralleled those in breast milk (data not shown).

Table 2 Breast milk fatty acid composition (weight percent of total fatty acids, wt%) of atopic and non-atopic mothers; group means (s.e.m.) and differences (with 95% confidence limits, CL)

	Atopic mothers (n=20),[a] wt% (s.e.m.)	*Non-atopic mothers (n=20), wt% (s.e.m.)*	*Difference, wt% (95% CL)*
LA 18:2n-6	14.04 (0.52)	15.21 (0.94)	−1.18 (−3.36, 1.01)
GLA 18:3n-6	0.10 (0.01)	0.10 (0.01)	0.00 (−0.02, 0.02)
DGLA 20:3n-6	0.39 (0.02)	0.43 (0.03)	−0.05 (−0.12, 0.03)
AA 20:4n-6	0.39 (0.01)	0.46 (0.03)	−0.07 (−0.13, −0.01)
Σn-6LCP[b]	0.98 (0.03)	1.10 (0.06)	−0.12 (−0.27, 0.02)
LA/Σn-6LCP	14.64 (0.68)	14.64 (1.16)	0.00 (−2.74, 2.73)
ALA 18:3n-3	1.00 (0.08)	1.02 (0.07)	−0.01 (−0.22, 0.20)
EPA 20:5n-3	Trace	Trace	
DHA 22:6n-3	0.22 (0.02)	0.29 (0.04)	−0.07 (−0.16, 0.03)
Σn-3LCP[c]	0.45 (0.03)	0.58 (0.09)	−0.13 (−0.32, 0.05)

[a]Mean of two measurements with 1 week in between.
[b]Sum of all LCP derivatives except direct elongation products of LA (18:3, 20:3, 20:4, 22:4 and 22:5 n-6).
[c]Sum of all LCP derivatives except direct elongation products of ALA (18:4, 20:4, 20:5, 22:5 and 22:6 n-3).
ALA = alpha-linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid.

Table 3 Breast milk fatty acid composition (weight percent of total fatty acids) of atopic mothers, before and after 1 week of supplementation with borage oil; mean levels (s.e.m.) and differences (95% confidence limits, CL) are shown

	Before supplementation,[a] wt% (s.e.m.)	*After supplementation, wt% (s.e.m.)*	*Difference, wt% (95% CL)*
Low-dose group[b]			
LA 18:2n-6	13.90 (0.69)	14.20 (0.67)	+0.30 (−1.02, +1.63)
GLA 18:3n-6	0.11 (0.00)	0.16 (0.01)	+0.06 (+0.03, +0.08)
DGLA 20:3n-6	0.41 (0.03)	0.53 (0.06)	+0.12 (+0.06, +0.19)
AA 20:4n-6	0.40 (0.02)	0.41 (0.03)	+0.01 (−0.03, +0.04)
Σn-6LCP[c]	1.02 (0.05)	1.19 (0.08)	+0.17 (+0.08, +0.26)
LA/Σn-6LCP	13.92 (0.94)	12.29 (0.79)	−1.62 (−2.59, −0.67)
High-dose group[b]			
LA 18:2n-6	14.17 (0.81)	14.16 (1.12)	−0.01 (−1.47, +1.44)
GLA 18:3n-6	0.09 (0.01)	0.18 (0.01)	+0.10 (+0.08, +0.11)
DGLA 20:3n-6	0.36 (0.03)	0.51 (0.03)	+0.15 (+0.11, +0.19)
AA 20:4n-6	0.38 (0.02)	0.38 (0.01)	−0.01 (−0.02, +0.01)
Σn-6LCP[c]	0.93 (0.04)	1.16 (0.04)	+0.23 (+0.17, +0.28)
LA/Σn-6LCP	15.36 (0.97)	12.17 (0.75)	−3.19 (−5.08, −1.30)

[a]Mean of two baseline measurements with 1 week in between.
[b]Borage oil supplement contained gamma-linolenic acid 230 mg/day (low-dose group) or 460 mg/day (high-dose group).
[c]Sum of all LCP derivatives except direct elongation products of LA (18:3, 20:3, 20:4, 22:4 and 22:5 n-6).

Discussion

We found that breast milk level of n-6 LCPs, notably AA, are lower in atopic mothers compared to non-atopic mothers. However, the ratio of LA to its LCP derivatives shows no differences between these groups. Since this ratio reflects the D6D enzyme activity, the results do not confirm an impaired activity of the enzyme delta-6-desaturase, which has been suggested to be the underlying mechanism of disturbances of LCP levels in atopy (Manku *et al*, 1982; Melnik & Plewig, 1992).

Three earlier studies have reported on breast milk fatty acids in atopic and non-atopic mothers. Schroten *et al* (1992) found a fatty acid pattern in breast milk that was almost identical for a group of mothers with allergic rhinoconjunctivitis or allergic bronchial asthma ($n=23$), a group of mothers with atopic eczema ($n=20$), and a control group of non-atopic mothers ($n=29$). Kohn *et al* (1995) reported on results of 31 atopic mothers and 31 non-atopic mothers, stating that breast milk LA-levels, the ratio of LA to GLA and the ratio of LA to GLA + DGLA + AA did not differ between these groups. Yu *et al* (1998) showed that DGLA was lower in 17 atopic than in 17 non-atopic mothers in milk samples obtained after 1 month of lactation; other differences were lower n-3 LCPs (eicosapentaenoic acid (20:5n−3), docosapentaenoic acid (22:5n-3) and docosahexaenoic acid (22:6n-3)), a higher ratio of total n-6 to n-3 LCPs in milk, and lower levels of the monounsaturated fatty acids. In addition, the same authors found that individual fatty acids from the n-6 series correlated with each other in 29 non-atopic subjects but did not correlate in 29 atopic subjects (Duchen & Bjorksten, 1998). To summarize, some but not all studies found an altered LCP pattern in breast milk of atopic mothers, but no study confirmed a disturbed conversion from LA to GLA. A first explanation for the inconsistencies between these studies may be related to the timing of breast sampling. Although fat content in mature breast milk varies by the duration of breastfeeding and during the day (Harzer *et al*, 1983), and during a nursing (Jensen *et al*, 1992; Hall, 1979), the fatty acid composition of the lipid fraction remains fairly constant in these time aspects (Jensen *et al*, 1992; Hall, 1979; Lammi-Keefe *et al*, 1990). Therefore, we do not believe that differences between studies could be explained by differences in the timing of breast milk sampling. Second, inconsistencies may be due to the definition of atopy and the severity of symptoms at the time of the study. No systematic agreement appears between the results of these studies and their clinical or laboratory criteria for atopy. Most mothers in our study were currently symptomatic, but some were (almost) symptom free (especially those with seasonal symptoms and effective treatment). We were not strict in including only symptomatic mothers, because the hypothesis on the association between essential fatty acids (EFA) and atopy implies that EFA is an underlying metabolic condition (and treatment is influencing pathways that are more distal to EFA metabolism, so that effective treatment cannot be expected to influence EFA status).

Previously, it has been suggested that low levels for n-6 LCPs in breast milk play a causal role in the development of atopy in the child (Melnik & Plewig, 1989). This was based on studies that have found decreased levels of n-6 LCP derivatives in umbilical cord blood of children at risk of atopy (Strannegård *et al*, 1987; Ioppi *et al*, 1994) and children who later developed atopy (Galli *et al*, 1994). Businco *et al* (1993) and Wright & Bolton (1989) showed that mothers with atopic children have lower levels of n-6 LCPs in their breast milk. Because LCPs have now been shown to be altered in the breast milk of atopic mothers, the hypothesis of a causal role can be further delineated. We propose that lowered LCP supply to the fetus and breastfed infant of atopic mothers is an additional risk factor for atopy in the child, especially if the underlying metabolic disturbance would also be inherited by the child. Such a perinatal maternal influence may also explain why the risk of atopy is higher in children with atopic mothers than in children with atopic fathers (Ruiz *et al*, 1992; Hide *et al*, 1994; Happle & Schnyder, 1982).

Because the conversion of LA to GLA is the rate limiting step in the series of conversions to DGLA and AA, supplementation of GLA in infancy has been suggested as a possible way to prevent atopy in the infant (Melnik & Plewig, 1989). We evaluated the possibility of supplementing lactating atopic mothers in order to restore decreased n-6 LCPs in their breast milk. We found that supplementation of borage oil for 1 week significantly raised the levels of GLA, DGLA and the sum of n-6 LCPs in breast milk in a linear dose-dependent way. No increase was found in the level of AA. Our results are in accordance with previous supplementation studies in healthy subjects. Administration of either evening primrose oil or blackcurrant seed oil (both rich in GLA) raised the levels of GLA and DGLA (Rassias & Gibson, 1988), and supplementation with evening primrose oil for 2−6 months increased the breast milk level of GLA and DGLA, but not AA (Cant *et al*, 1991). Therefore, it is unlikely that the absence of an increase in AA in our study is due to the short duration of supplementation or to a disturbance of fatty acid metabolism related to atopy of the mother. We conclude that GLA supplementation of the atopic mother does not restore the lower AA level in her breast milk compared to non-atopic mothers.

To summarize, we showed decreased levels of n-6 LCPs, mainly AA, in atopic mothers; but we did not confirm that this is due to a disturbed D6D activity. The decreased AA level cannot be restored by GLA supplementation of the mother's diet. At present, there is insufficient ground for recommending GLA supplementation to atopic lactating mothers.

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